

Free-living nitrogen fixers and acetylene reduction in tropical rice field

In order to assess the contribution of microbial nitrogen fixation to nitrogen fertility of paddy fields in Thailand, the abundance of free-living nitrogen fixers (dilution method) and nitrogen-fixing activities (acetylene reduction method) were investigated for topsoil (0–2 cm) and paddy water samples consecutively taken at 40 paddy fields throughout a rice-growing season.

Although nitrogen-fixing populations and acetylene-reducing activities more or less fluctuated during one cropping season, they were apt to reach the maximum at tillering to heading stages.

As in Fig. 1, correlations were obtained between the annual average populations and soil chemical properties. Soil rich in organic matter supported better growth of heterotrophs (*Azotobacter* and *Clostridium butyricum*), whereas soils rich in phosphorus generally stimulated the growth of the heterotrophs and blue-green algae. Growth of all nitrogen fixers examined were highly correlated to soil pH within 4–8.

Furthermore, higher populations of *C. butyricum* and blue-green algae were counted in soils rich in available nitrogen, suggesting that these two microbial groups mainly contribute to enrichment of soil nitrogen.

The influences of soil properties on the growth of nitrogen fixers seem responsible for building up of nitrogen-fixing microflora characteristic to each paddy soil group. As

Table 1. Characterization of each paddy soil group in Thailand by nitrogen-fixing microflora and nitrogen fixation in the field

Soil group*	Habitat	Nitrogen-fixing microflora**					N fixation N kg/ha/year range (mean)
		<i>Azotobacter</i>	<i>Beijerinckia</i>	<i>Clostridium butyricum</i>	Nonsulfur purple bacteria	Blue green algae	
MA	Water Soil	<10 ¹	—	10 ² –10 ³	10 ¹ –10 ²	10 ¹ –10 ²	1.3–17.4 (9.3)
		1–10 ³	—	10 ⁴ –10 ⁵	10 ³ –10 ⁴	10 ¹ –10 ⁴	
BWA	Water Soil	<1	—	10 ² –10 ³	10 ¹	<10 ¹	2.7–4.3 (3.4)
		≤10 ¹	—	10 ⁴ –10 ⁵	10 ² –10 ³	10 ²	
FWA	Water Soil	<10 ²	—	10 ² –10 ⁴	10 ¹ –10 ³	10 ¹ –10 ³	4.2–53.9 (17.5)
		10 ¹ –10 ⁴	≤10 ¹	10 ⁴ –10 ⁶	10 ³ –10 ⁵	10 ³ –10 ⁴	
LHG-I	Water Soil	<1	—	10 ² –10 ³	10 ¹ –10 ²	<10 ¹	0.5–3.9 (2.1)
		<10 ¹	<10 ¹	10 ³ –10 ⁴	10 ³ –10 ⁴	10 ² –10 ³	
LHG-II	Water Soil	<10 ¹	—	10 ² –10 ³	10 ¹ –10 ³	<10 ²	2.4–7.4 (4.2)
		10 ¹ –10 ²	<10 ¹	10 ²	10 ³ –10 ⁵	10 ³ –10 ⁴	
LHG-III	Water Soil	<10 ¹	—	10 ² –10 ⁴	10 ¹ –10 ³	10 ² –10 ³	2.0–9.9 (5.1)
		10 ¹ –10 ²	≤10 ¹	10 ²	10 ³ –10 ⁴	10 ³ –10 ⁴	
HG	Water Soil	10 ¹	—	10 ³	10 ¹	10 ³	16.0
		10 ⁴	—	10 ⁶	10 ³	10 ⁴	
NCB	Water Soil	<10 ¹	—	10 ³	10 ³	10 ²	4.4
		10 ³	<10 ¹	10 ⁵	10 ³	10 ³	
GP	Water Soil	10 ²	—	10 ³	10 ³	10 ⁴	4.6
		10 ³	—	10 ⁵	10 ⁴	10 ⁴	
G	Water Soil	10 ³	—	10 ³	10 ²	10 ³	8.3
		10 ³	—	10 ⁵	10 ³	10 ⁴	
R	Water Soil	<1	—	10 ¹	10 ¹	<1	0.7
		10 ¹	—	10 ³	10 ²	10 ²	

* MA: Marine Alluvial Soil, BWA: Brackish Water Alluvial Soil, FWA: Fresh Water Alluvial Soil, LHG: Low Humic Gley Soil, HG: Humic Gley Soil, NCB: Noncalic Brown Soil, GP: Gray Podzolic Soil, G: Grumusol, R: Regosol

** annual average population per 1 g of dry soil

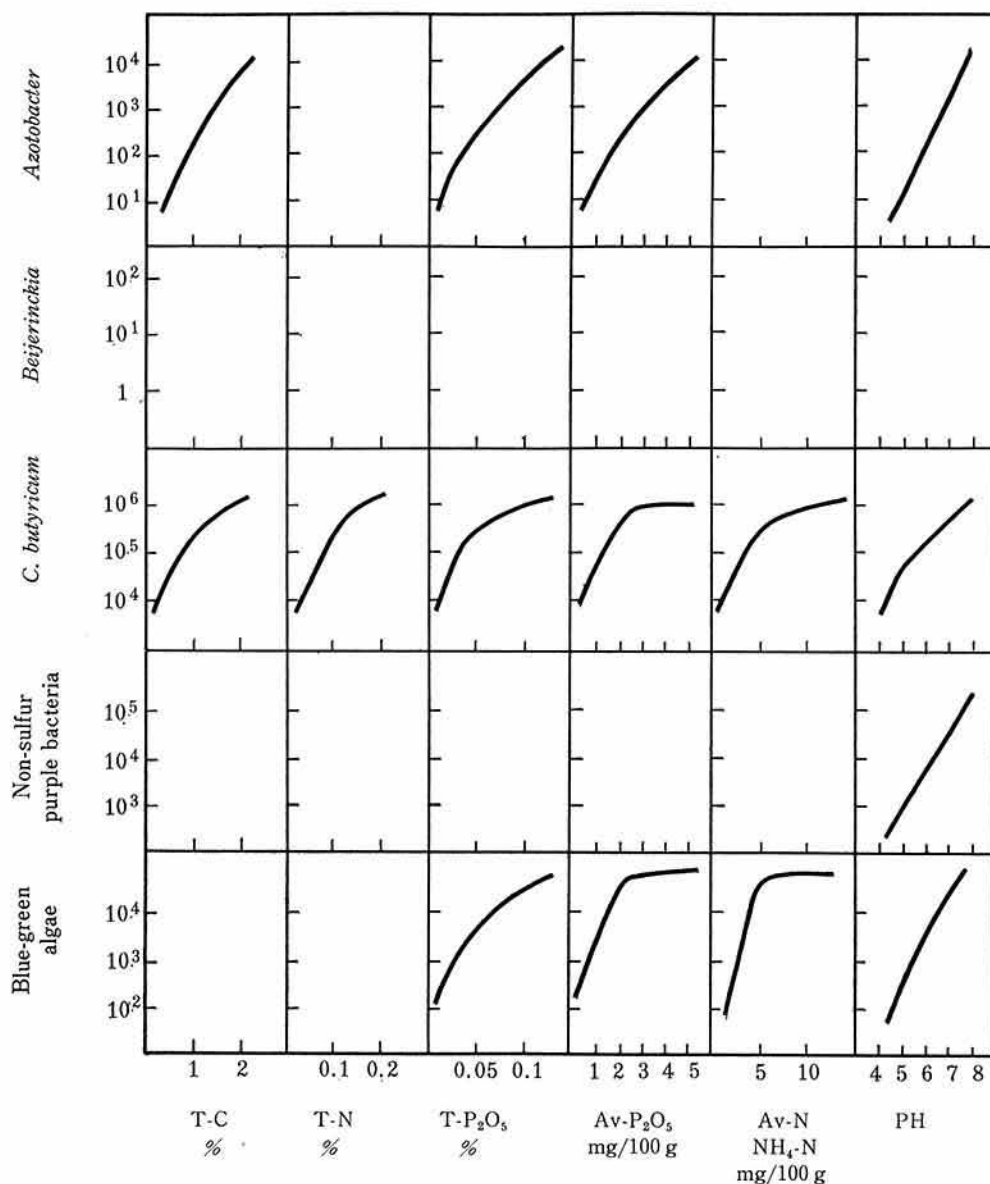


Fig. 1. Relationships between N-fixing population and some chemical properties of plough soil

in Table 1, Marine Alluvial Soil, Fresh Water Alluvial Soil, Humic Gley Soil and Grumusol were relatively abundant in *Azotobacter*, *C. butyricum* and blue-green algae, whereas acid sulfate soils of Brackish Water Alluvium and Acid Sandy Regosol were poor in all nitrogen-fixing microbes.

Among Low Humic Gley Soil, the growth of *Azotobacter*, *C. butyricum* and blue-green

algae were poor in the soils with less organic matter (group I), while they were stimulated in the soils with more organic matter (groups II and III). Noteworthy in this soil group was the highest population of non-sulfur purple bacteria among all soil groups.

Acetylene-reducing activities of soil considerably corresponded to the abundance of nitrogen fixers.

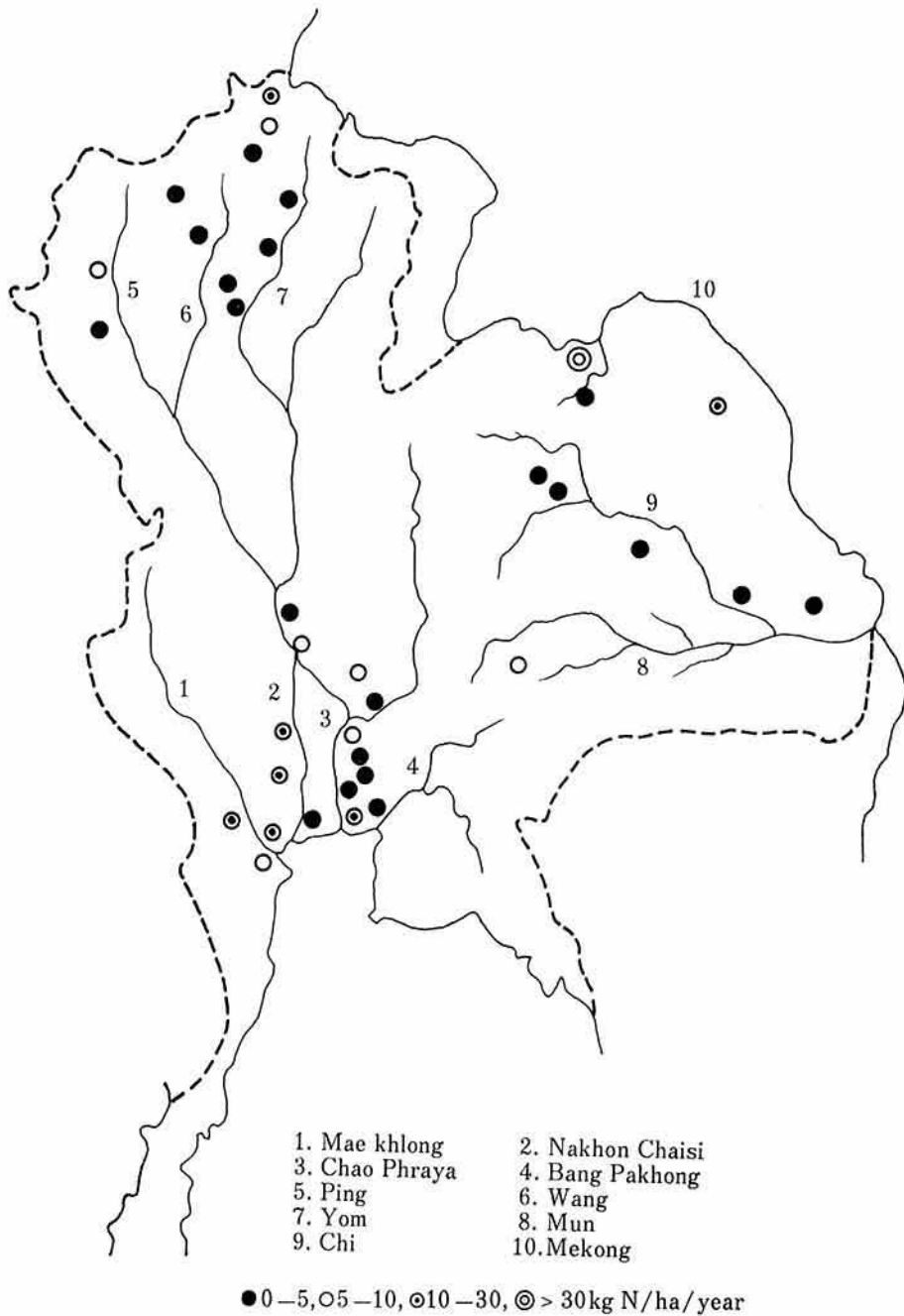


Fig. 2. Geographic distribution of microbial N-fixing capacity of paddy field in Thailand

Amounts of annual nitrogen fixation in the field were estimated from acetylene-reducing activities of soil and paddy water. Most of the paddy fields in Thailand were revealed to

fix less than 10 kg N/ha, though some of the Marine Alluvial Soil and Fresh Water Alluvial Soil fixed around 20 kg N/ha. The average in the whole country was 6.9 kg N/ha. This

amounts as low as 20% of the total nitrogen annually taken up by growing rice, suggesting that, in Thailand, nitrogen fixation in rice rhizosphere and in irrigation ditch must play an appreciable part in natural nitrogen supply to paddy field.

As indicated in Fig. 2, the fields in the basins of the Chao Phraya, Ping, Wang, Yom and Chi rivers fixed less than 5 kg N/ha, while those close to the Mae Klong, Nakhon Chaisi and Mekong rivers were shown to fix as much as 10 to 30 kg N/ha. The factors of paddy soil formation, including irrigation water quality, must be in close relation to the establishment of soil nitrogen-fixing society.

In order to increase microbial nitrogen fixation in the paddy fields of Thailand, improvement of soil properties, especially of acidic soil reaction of Brackish Water Alluvial Soil and Low Humic Gley Soil and the paucity in phosphorus availability of most soil groups, is indispensable.

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Microflora related to the nitrogen cycle in paddy soils of Thailand

In the nitrogen cycle of the soil, microorganisms play an important role such as nitrogen fixation, mineralization of organic matter, denitrification, immobilization of soil nitrogen and each of these processes depends upon the action of microorganisms of various groups.

On the other hand, each soil has its own chemical and physical characteristics being disposed under the different climatic condition. Therefore, microbiological condition is different according to the kinds of soils. And it was recognized that each soil has the char-

acteristic microflora and its activity^{1),2)}, while there is scarce information about microflora of tropical paddy soils^{3),4),5)}.

In order to clarify the microbiological character of tropical paddy soils, microflora related to the nitrogen cycle were examined systematically with the soil samples collected as a set of the top soil (0-1 cm) and sub-surface soil (1-10 cm) from 98 paddy fields twice in the mid-rainy and mid-dry seasons.

(1) Difference of microflora in typical great soil groups of Thailand

Each great soil group showed the characteristic microflora, which differs by the seasons. Aerobic bacteria were found abundantly in Fresh Water Alluvial Soils and were less in Brackish Water Alluvial Soils except in the top soil in the rainy season. Anaerobic bacteria were also abundant in Fresh Water Alluvial Soils. A small number of actinomycetes was found in Brackish Water Alluvial Soils and this is ascribable to the low pH value of this soil because actinomycetes is very weak at pH value below 4.0.

The data obtained hitherto show that nitrosobacter is mostly 10 times of the number of nitrite oxidizer but the number of nitrosobacter in Thailand's paddy soils was not high in comparison with that of the nitrite oxidizer.

The average number of nitrosobacter showed $4.08-0.13 \times 10^3$ and the maximum value of the average number was found in the Fresh Water Alluvial Soils, while that of nitrite oxidizer was $74.8-0.34 \times 10^3$ and the maximum value was found in Low Humic Gley Soils in the top soil and the Marine Alluvial Soils in the sub-surface soil.

In the rainy season, denitrifiers in the Fresh Water Alluvial and Low Humic Gley Soils were higher in number ($1,100-564 \times 10^3$) than those in the Brackish Water Alluvial and Marine Alluvial Soils ($369-132 \times 10^3$) irrespective of soil layers, whereas those in the Brackish Water Alluvial Soils in the dry season showed very high value ($1,301-183 \times 10^3$), which were supposed to be due to the high organic matter content.

Table 1. Average number of each micro-organism related to the nitrogen cycle in each soil group in respect of seasons and soil layers ($\times 10^3$ per dry soil 1 g)

Soil group	Rainy Season		Dry Season	
	0-1 cm	1-10 cm	0-1 cm	1-10 cm
(1) Ammonifier				
(A)*	109	62.3	14.0	19.2
(B)*	151	72.6	28.1	9.02
(C)*	32.0	307	0.59	0.91
(D)*	11.3	68.3	2.53	1.63
(2) Nitrosobacter				
(A)	2.41	1.65	0.68	0.28
(B)	3.86	4.08	0.87	1.87
(C)	1.55	0.12	0.22	1.94
(D)	0.13	0.052	0.15	0.13
(3) Nitrite Oxidizer				
(A)	74.8	13.8	1.47	1.56
(B)	19.7	11.6	3.78	2.74
(C)	2.68	0.34	1.11	6.79
(D)	1.35	0.34	0.90	8.11
(4) Denitrifier				
(A)	922	564	193	169
(B)	1,100	559	119	90.3
(C)	134	132	1,301	183
(D)	124	369	17.3	17.3
(5) Cellulose decomposer				
(A)	19.4	18.0	50.8	26.7
(B)	50.7	24.8	51.6	51.8
(C)	10.4	8.3	10.4	14.1
(D)	9.5	19.9	7.4	5.45

(A)* Low Humic Gley Soils

(B)* Fresh Water Alluvial Soils

(C)* Brackish Water Alluvial Soils

(D)* Marine Alluvial Soils

The order of the numbers of cellulose decomposer showed the maximum value in the Fresh Water Alluvial Soils and the minimum one, in Marine Alluvial Soils in the top soils in both the rainy and dry seasons, while in the sub-surface soil, high values were found in the Fresh Water Alluvial and Marine Alluvial Soils in the rainy season and the number of cellulose decomposer in the Fresh Water Allu-

vial and Low Humic Gley Soils were higher than those in the Marine and Brackish Water Alluvial Soils in the dry season. The order of the number of cellulose decomposer corresponded completely with their activities.

(2) Difference of microflora between the rainy and dry seasons

In general, the numbers of microorganisms were mostly higher in the rainy season than in the dry season, though there were some exceptions.

As to the exceptions, the numbers of ammonifier in the Brackish Water Alluvial Soils in both the top and sub-surface soils showed far lower values in the dry season than in the rainy season.

The numbers of denitrifier in the Brackish Water Alluvial Soils were higher in the dry season irrespective of soil layers.

The numbers of nitrosobacter and nitrite oxidizer in the sub-surface soils in both the Brackish Water and Marine Alluvial Soils were higher in the dry season.

(3) Difference between the top and sub-surface soils

In general, the number of each micro-organism was generally higher in the top soil than in the sub-surface soil in the rainy season, but in the dry season, non-spore forming groups such as some anaerobic bacteria, ammonifiers and nitrite oxidizer were found higher in number in the sub-surface soil than in the top soil.

The number of ammonifier in Brackish Water Alluvial Soils was far higher in the sub-surface soil than in the top soil regardless of the low number of aerobic bacteria in the sub-surface soil in the rainy season.

In comparison with Japanese data on paddy soils, the numbers of actinomycetes and denitrifier in Thailand were extremely high while aerobic bacteria and nitrifier showed low values.

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